Basis for Prokaryotic Selectivity of the Antibiotic Paromomycin

Brookfield Academy SMART Team

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Ribosomes are responsible for protein synthesis and are major targets of antibiotics. While translation is a universally conserved cellular process, the ability of drugs to target prokaryotic ribosomes depends on subtle variations from eukaryotic ribosomes. The ribosome is composed of ribosomal RNA (rRNA) and protein. The small ribosomal subunit, called 30s in prokaryotes, contains 21 proteins and one rRNA (16S) while the large subunit, called 50S, contains 31 proteins and two rRNAs (23S and 5S). Recent crystal structures reveal that the rRNAs adopt a 3D fold generating (I) decoding center for codon-anticodon recognition, (II) a peptidyl-transfer center (PTC) for a peptide bond formation and (III) an exit tunnel through which the nascent protein emerges. The decoding center is one of the potential targets for anti-bacterial drug development. For example, paromomycin, used in the treatment of intestinal infections, selectively inhibits prokaryotic ribosomes at the decoding site. Paromomycin physically interacts with the helix H44 (formed as a result of coupling between 16S rRNA nucleotides 1400-to-1420 and 1480-to-1500) and prevents proper rotation of A1492 and A1493 during anticodon:codon recognition, thus decreasing tRNA selection accuracy in prokaryotic ribosomes. However, paromomycin fails to affect eukaryotes due to an A to G transition at position 1408. The Brookfield Academy SMART Team (Students Modeling A Research Topic) modeled a prokaryotic ribosome, highlighting nucleotides responsible for the prokaryotic specificity of paromomycin.

MANY ANTIBIOTICS, INCLUDING PAROMOMYCIN, TARGET BACTERIAL RIBOSOMES AND INTERFERE WITH PROTEIN SYNTHESIS

Bacteria are the cause of many diseases. Normally our bodies fight these infections, but sometimes assistance is necessary through prescription antibiotics. Antibiotics kill or inhibit the growth of bacteria by interfering with enzymes or processes specific to bacterial function. For instance, many antibiotics target bacterial cell wall synthesis, while others inhibit protein synthesis by prokaryotic ribosomes. Aminoglycoside antibiotics, such as paromomycin, target the ribosome within bacteria. Unlike antibiotics that target enzymes involved in cell wall synthesis, antibiotics that target ribosomes are effective against both Gram-positive and Gram-negative bacteria. In addition, paromomycin was licensed in India as an effective treatment against visceral leishmaniasis (also known as the black fever). Visceral leishmaniasis is the second-largest parasitic killer in the world, outmatched only by malaria; it is responsible for over 500,000 infections every year.

The ribosome serves the cell as a protein making machine. Its complex methods of protein synthesis cause it to an efficient natural nano-machine. Protein formation occurs in three steps: initiation, elongation, and termination. Paromomycin interfers with proper protein synthesis in prokaryotic ribosomes by preventing incorporation of the correct amino acid during elongation of the polypeptide chain.

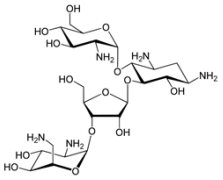

Figure 1. Structure of the antibiotic paromomycin.

RIBOSOMES DECODE THE GENETIC INFORMATION INTO A POLYPEPTIDE CHAIN

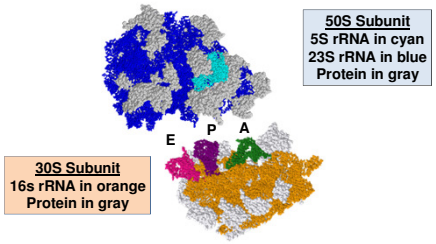

Figure 2. Surface representation of crystal structures of ribosomal subunits 30S and 50S. The small subunit (30S) has been crystalized with three tRNAs colored in pink, violet and green; and located at A (Aminoacyl-tRNA binding site), P (Peptidyl-tRNA binding site), and E (Exit site) sites. Figure generated using JMOL and PDB files 2XQE and 2XQD.

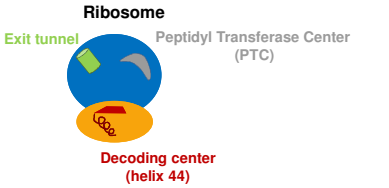

Figure 3. Overall, the 2 subunits assemble to generate (I) decoding center for codon-anticodon recognition, (II) a peptidyl-transfer center (PTC) for a peptide bond formation and (III) an exit tunnel through which the nascent protein emerges. Helix 44 at the A site of the 30S subunit supports the interaction between the codon of mRNA and the cognate anticodon of the aminoacyl-tRNA. PTC of 50S subunit catalyzes peptide bond formation between amino acids.

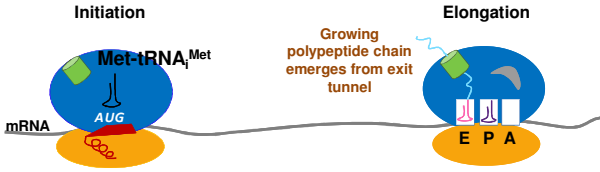

Figure 4. Ribosome, **initiator methionyl tRNA (Met-tRNA$_i^{Met}$)** and other initiation factors (not shown here) assemble on the start codon ***AUG*** of mRNA. During this assembly, the **Helix 44** plays a crucial role in sub-unit joining and stabilizing the codon-anticodon interaction, which prevents an incorrect amino acid from being incorporated into protein.

Figure 5. Elongating ribosome with E, P and A sites. Amino acids bound to new tRNAs enter the A site, where only tRNA with the correct anticodon pairs with the mRNA codon at this site. A correct anticodon:codon pair, triggers peptide bond synthesis between the growing peptide chain at the P site, and the incoming amino acid of the A site tRNA.

Figure 6. Ribosomal subunits dissociate when encountered with one of three stop codons (**UAG**, **UUG** or **UGA**) and the nascent polypeptide chain is released.

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PAROMOMYCIN INTERFERES WITH AMINO ACID MATCHING ACTIVITY OF THE 30S SUBUNIT

Figure 7. A network of interactions between an-mRNA codon (purple), anticodon of A-tRNA (green) and Helix 44 (yellow). RNA backbones are shown as ribbons and bases as slab representations. These functional interactions require movement of nucleotides A1492 and A1493 (orange) located in helix H44 of the 16S rRNA, as illustrated by dotted arrows. Figure taken from Gregor et al.

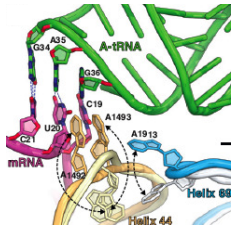
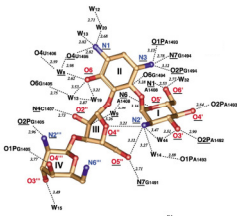
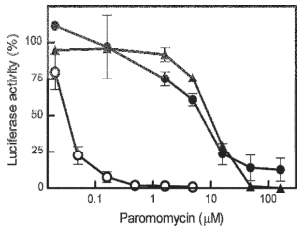


Figure 8. The binding pocket of paromomycin to the A site of the 30S ribosome. The polar contacts (H-bond contacts, relative distance < 2.8 Å) with rRNA nucleotides are shown as dotted lines. Binding of paromomycin causes nucleotides A1492 and A1493 to contact the codon--anticodon base pair, regardless of proper base pair matching. This leads to improper amino acid incorporation into the nascent peptide. Figure from Vicens and Westhof.



PAROMOMYCIN INHIBITS PROKARYOTIC BUT NOT EUKARYOTIC RIBOSOMES DUE TO rRNA SEQUENCE DIFFERENCES in HELIX 44 of 30S SUBUNIT

Figure 9. Effect of paromomycin on protein synthesis in bacterial ribosomes (o) versus human-bacterial hybrid ribosomes (●), as compared to eukaryotic ribosomes (▲). Translation efficiency was measured by the ability of ribosomes to translate a luciferase mRNA in cell-free translation assays. Paromomycin inhibits synthesis of luciferase in bacterial ribosomes but not in bacterial-human hybrid ribosomes in which helix 44 of bacterial ribosome has been replaced with eukaryotic helix 44 rRNA sequence containing G1408. Data taken from Sven et al.



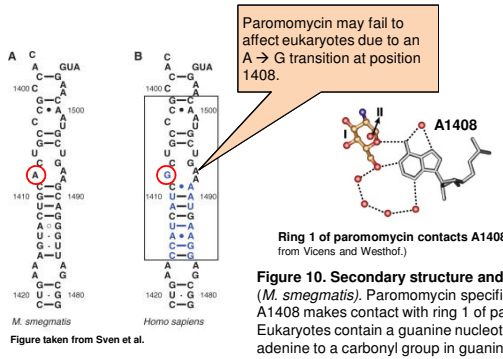
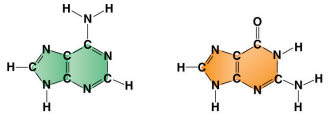


Figure taken from Sven et al.

Ring 1 of paromomycin contacts A1408. (Figure from Vicens and Westhof.)



Chemical structures are different between adenine and guanine. (Figures from Pearson Publishing)

Figure 10. Secondary structure and nucleotide composition of the helix 44. (A) The helix 44 of bacteria (*M. smegmatis)*. Paromomycin specificity for bacterial ribosome may be due to presence of A1408 (red circled). A1408 makes contact with ring 1 of paromomycin. (B) The helix 44 of human (*Homo sapiens)* ribosome. Eukaryotes contain a guanine nucleotide at the corresponding position of A1408. The change from an amine in adenine to a carbonyl group in guanine, may result in the loss of specific H-bond contacts required for tight binding of paromomycin to the prokayotic ribosome.

UNDERSTANDING STRUCTURAL DIFFERENCES BETWEEN PROKARYOTIC AND EUKARYOTIC RIBOSOMES MAY GUIDE NEW DRUG DESIGN

	Prokaryotes	Eukaryotes	Archaea
Small Subunit	**30S**	**40S**	**30S**
rRNA	**16S**	**18S**	**16S**
Number of proteins	**20**	**32**	**28**
Large Subunit	**50S**	**60S**	**50S**
rRNA	**5S, 23S**	**5S, 5.8S, 28S**	**5S, 23S**
Number of proteins	**34**	**46**	**40**

The ribosome is an existential part of life in that it synthesizes thousands of proteins needed to carry out life processes. Antibiotics, which generally affect ribosomal activity, are often used in treating bacterial diseases such as strep throat. The primary architecture of the ribosomes in bacteria, archaea, and eukaryotes is quite similar. However, ribosomes differ in size, nucleotide sequence, and the RNA-protein ratio, as indicated in the table at left. These differences allow for the design of prokaryotic-specific antibiotics, such as paromomycin. Continued understanding of how nucleotide sequence and structural differences impact ability of antibiotics to target and inhibit prokaryotic ribosomes may lead to development of future antimicrobial drugs.

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